?b 411
27sep99 14:14:03 User231835 Session D509.1
?sf all
?s (saep? and (lps or endotoxin? or lipopolysaccharide?))
?s (saep? and (lps or endotoxin? or lipopolysaccharide?))
S1 15 (SAEP? AND (LPS OR ENDOTOXIN? OR LIPOPOLYSACCHARIDE?))
?t s2/3,kwic/1-6
2/3,KWIC/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11266727 BIOSIS NO.: 199800048059
A model of Neisseria meningitidis vaccine based on *LPS* micelles detoxified by synthetic anti-*endotoxin* peptides.

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JOURNAL: Journal of Endotoxin Research 4 (4):p261-272 Aug., 1997
ISSN: 0968-0519
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

A model of Neisseria meningitidis vaccine based on *LPS* micelles detoxified by synthetic anti-*endotoxin* peptides.

ABSTRACT: We describe a model of vaccine based on detoxified *endotoxin* (*LPS*) conserving the supramolecular structure of micelles. Detoxification of *LPS* from Neisseria meningitidis group A, strain A1 (*LPS* A1), has been achieved by complex formation with a synthetic anti-*endotoxin* peptide (*SAEP* 2) binding to the lipid A moiety of *LPS* A1 with high affinity. Following subcutaneous injection in SW mice, *LPS* A1/*SAEP* 2 complex induced high titers of boostable IgG antibodies against the immunotype determinants of *LPS* A1, cross-reactive with group B *LPS* in either purified or cell-associated form. These antibodies were able to functionally fix and activate homologous and heterologous species of complement after binding to *LPS* A1-coated sheep erythrocytes. None of the IgG antibodies induced were specific for lipid A or *SAEP* 2 and none of the IgG antibodies cross-reacted with heterologous *LPS*. The purified IgG polyclonal antibodies significantly inhibited serum TNF production in CD1 mice intravenously challenged by homologous but not heterologous *LPS*. The immunogenic properties of *LPS* A1/*SAEP* 2 complex, investigated by the kinetic, magnitude and sub-isotype composition of the polyclonal antibodies induced, were comparable to those of a glycoconjugate obtained by covalent binding of *LPS* A1, detoxified by *SAEP* 2, to BSA working as a T-cell dependent carrier protein. The results obtained suggest that *LPS* behaves in vivo as a T-cell dependent antigen. The strategy of properly delivering to the immune system of mammalians, non-toxic *LPS* fully expressing its supramolecular antigenic structure, represents a novel approach for development of a new generation of R- and S-*LPS*/*SAEP* complex-based vaccines for prophylaxis of specific Gram-negative infections leading to sepsis and endotoxemia.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: anti-*endotoxin* peptides...

...*lipopolysaccharide* micelles

2/3,KWIC/2 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05535851 Genuine Article#: WF094 No. References: 32
Title: Inhibition of *LPS*-induced systemic and local TNF production by a syntheticanti-*endotoxin* peptide (*SAEP*-2)
Author(s): Demitri MT; Velucchi M; Bracci L; Rustici A; Porro M (REPRINT); Villa P; Ghezzi P
Corporate Source: ZORA IND LOC SENTINO,/I-53040 RAPOLANO TERNE/SIENA/ITALY/ (REPRINT); IST RIC FARMACOL MARIO NEGRI,/MILAN//ITALY/; CNR,CELLULAR & MOL PHARMACOL CTR/I-20133 MILAN//ITALY/; UNIV SIENA,DEPT BIOL MOL/I-53100 SIENA//ITALY/; BIOSYNTH SRL,/SIENA//ITALY/
Journal: JOURNAL OF ENDOTOXIN RESEARCH, 1996, V3, N6 (DEC), P445-454
ISSN: 0968-0519 Publication date: 19961200
Publisher: CHURCHILL LIVINGSTONE, JOURNAL PRODUCTION DEPT, ROBERT STEVENSON HOUSE, 1-3 BAXTERS PLACE, LEITH WALK, EDINBURGH, MIDLOTHIAN, SCOTLAND EH1 3AF
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

Title: Inhibition of *LPS*-induced systemic and local TNF production by a syntheticanti-*endotoxin* peptide (*SAEP*-2)
Abstract: *Lipopolysaccharide* (*LPS*) exerts its biological activity through the lipid A moiety. We tested the efficiency in inhibiting...
...production in sera andin tissues of mice and in the derma of rabbits challenged with *LPS*, of a synthetic anti-*LPS* peptide (*SAEP*-2) previously shown to specifically detoxify the lipid A region of *LPS* on the basis of structural similarities with the antibiotic polymyxin B (PMXB). in mice, *SAEP*-2 (100 mu g/mouse, i.v.) injected with various schedules (-30 to +10 min from *LPS* at 50 ng/mouse, i.v.)significantly inhibited serum TNF as well as liver, spleen and lung-associated TNF. In rabbits, *SAEP*-2 significantly inhibited TNF produced in dermaltissue and the resulting local hemorrhagic necrosis. The amount of tissue-associated TNF released by *LPS* challenge in the mouse was up to 6 times that present in the serum and inhibition by *SAEP*-2 or PMXB accounted for 75% of the total. Direct measurement of the binding kinetics by surface plasmon resonance and molecular filtration at equilibrium revealed that *SAEP*-2 and PMXB bind to *LPS* only in the presence of a significant amount of water but that they are unable to bind *LPS* in undiluted serum. Altogether these findings strongly suggest that inhibition of *LPS*-induced TNF by *SAEP*-2 and PMXB may occur in tissues.
...Identifiers--TUMOR-NECROSIS-FACTOR; PERMEABILITY-INCREASING PROTEIN; POLYMYXIN-B; LIPID-A; BACTERIAL *LIPOPOLYSACCHARIDES*; BINDING-SITE; SEPTIC SHOCK; MICE; CACHECTIN; DETOXIFICATION

2/3,KWIC/3 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00540368 97040332
Inhibition of *LPS*-induced systemic and local TNF production by a synthetic anti-*endotoxin* peptide (*SAEP*-2)
Demitri M.T.; Velucchi M.; Bracci L.; Rustici A.; Porro M.; Villa P.; Ghezzi P.
ADDRESS: Dr. M. Porro, BiosYnth srl, Zona Industriale-Loc. Sentino, 53040 Rapolano Terme, Siena, Italy
Journal: Journal of Endotoxin Research, 3/6 (445-454), 1996, United Kingdom
PUBLICATION DATE: 19960000
CODEN: JENRE
ISSN: 0968-0519
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 32

Inhibition of *LPS*-induced systemic and local TNF production by a synthetic anti-*endotoxin* peptide (*SAEP*-2)

Lipopolysaccharide (*LPS*) exerts its biological activity through the lipid A moiety. We tested the efficiency in inhibiting...
...in sera and in tissues of mice and in the derma of rabbits challenged with *LPS*, of a synthetic anti-*LPS* peptide (*SAEP*-2) previously shown to specifically detoxify the lipid A region of *LPS* on the basis of structural similarities with the antibiotic polymyxin B (PMXB). In mice, *SAEP*-2 (100 mug/mouse, i.v.) injected with various schedules (-30 to +10 min from *LPS* at 50 ng/mouse, i.v.) significantly inhibited serum TNF as well as liver, spleen and lung-associated TNF. In rabbits, *SAEP*-2 significantly inhibited TNF produced in dermal tissue and the resulting local hemorrhagic necrosis. The amount of tissue-associated TNF released by *LPS* challenge in the mouse was up to 6 times that present in the serum and inhibition by *SAEP*-2 or PMXB accounted for 75% of the total. Direct

measurement of the binding kinetics by surface plasmon resonance and molecular filtration at equilibrium revealed that *SAEP*-2 and PMXB bind to *LPS* only in the presence of a significant amount of water but that they are unable to bind *LPS* in undiluted serum. Altogether these findings strongly suggest that inhibition of *LPS*-induced TNF by *SAEP*-2 and PMXB
may occur in tissues.

2/3,KWIC/4 (Item 1 from file: 348)
DIALOG(R)File 348:European Patents

00924810
ORDER fax of complete patent from Dialog SourceOne. See HELP ORDER 348
Combined use of anti-*endotoxin* synthetic peptides and of anti-*endotoxin* antibodies for the prophylaxis and treatment of endotoxicosis and septic shock
Kombinierte Verwendung von synthetischen Peptiden gegen *Endotoxin* und von
Antikorpern gegen *Endotoxin* zur Vorbeugung und Behandlung von Endotoxicosis und septis
Utilisation combinee des peptides synthetiques contre l'*endotoxine* et des anticorps contre l'*endotoxine* pour la prophylaxie et le traitement de l'endotoxicose e
PATENT ASSIGNEE:
BIOSYNTH S.R.L., (1911550), Zona Industriale Loc Sentino, I-53040 Rapolano Terme, (IT), (applicant designated states: AT;BE;CH;DE;DK;ES;FI;FR;GB;GR;IE;IT;LI;LU;MC;NL;PT;SE)
INVENTOR:
Porro, Massimo, Via Selvapiana, 97, 53040 Rapolano Terme (Siena), (IT)
LEGAL REPRESENTATIVE:
De Gregori, Antonella (87231), Ing. Barzano & Zanardo Milano S.p.A. Via Borgonuovo 10, 20121 Milano, (IT)
PATENT (CC, No, Kind, Date): EP 842666 A2 980520 (Basic)
APPLICATION (CC, No, Date): EP 97203526 971112;
PRIORITY (CC, No, Date): IT 96MI2354 961113
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU;
MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: A61K-039/40; A61K-038/06; A61K-039/40;
A61K-038/06
ABSTRACT WORD COUNT: 54

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:
Available Text Language Update Word Count
CLAIMS A (English) 9821 825
SPEC A (English) 9821 1774
Total word count - document A 2599
Total word count - document B 0
Total word count - documents A + B 2599

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Combined use of anti-*endotoxin* synthetic peptides and of anti-*endotoxin* antibodies for the prophylaxis and treatment of endotoxicosis and septic shock
Kombinierte Verwendung von synthetischen Peptiden gegen *Endotoxin* und von
Antikorpern gegen *Endotoxin* zur Vorbeugung und Behandlung von Endotoxicosis und septis
Utilisation combinee des peptides synthetiques contre l'*endotoxine* et des anticorps contre l'*endotoxine* pour la prophylaxie et le traitement de l'endotoxicose e

...ABSTRACT A2
Methods and compositions for neutralizing *endotoxin* and for the prophylaxis and treatment of endotoxicosis and septic shock are disclosed, which comprise the use of peptides specifically binding to the conserved *endotoxin* structure (Lipid A), and antibodies specifically binding to the antigenic determinants in the *endotoxin* core structure of different genera of Gram-negative bacteria.

SPECIFICATION Shock induced in mammals, particularly humans, by bacterial
endotoxins present in the blood and organs (endotoxemia) is also known as septic shock. This physiological...

...events. It is today well recognized that the agent responsible for this disease is bacterial *endotoxin*, which is a glycolipid antigen present only on the surface of Gram-negative bacteria. This glycolipid is also known as lipo-polysaccharide (*LPS*) or lipo-oligosaccharide (LOS) depending on the length of the saccharide chain which is covalently...

...to the disaccharide hydroxyls. Only Lipid A is responsible for the toxic effects of bacterial *endotoxins*, a fundamental characteristic being that its molecular structure is conserved between various *LPS*. When *LPS* is released into the blood stream by bacteria, specialized cells of the immune system like macrophages and macrocytes are activated by the *LPS* and several immune mediators are released (Cytokines such as Interleukin-1 and Interleukin-6; Tumor necrosis factor; Gammainterferon).

Furthermore, *endotoxin* also activates the complement cascade which results in cell lysis of *LPS*-activated cells, with the consequent release of proteolytic enzymes promoting the release of vasoactive peptides...

...are of some benefit.
Polymyxin B is a peptide antibiotic which binds and detoxifies bacterial *endotoxins* on the basis of its affinity for Lipid A. Polymyxin B can prevent the effects...

...Septic shock can be caused by infection with any bacteria that cause the release of *LPS*, including Pseudomonas Aeruginosa, Escherichia Coli, Salmonella Typhi, Neisseria Meningitidis, Neisseria Gonorrheae, Bordetella Pertussis, Klebsiella Pneumoniae...

...as to the possible binding to cell receptors structurally comparable to the Lipid A of *LPS* (the gangliosides of the neutral tissues are glycolipids with N,O-acyl (C14))-C18))) chains...

...acyl chains present in the Lipid A structure).
It is known that certain conformation peptides (*endotoxin* or *SAEP* binding peptides) although structurally different from Polymyxin B by virtue of their amino acid composition...

...capable of binding to the same binding site within the Lipid A of the LOS/*LPS* as Polymyxin B. These peptides are well tolerated by mammals on
the basis of their...

...within the organs and tissues, in contrast to Polymyxin B. The binding efficiency of certain *SAEPs* for Lipid A is comparable to the affinity constant value of Polymyxin B. The complex formed when Lipid A or *LPS* is reacted with these peptides is non-toxic and the natural antigenicity of Lipid A and *LPS* is maintained. These *SAEPs* can be monomers, linear
polymers, cyclic monomers or cyclic polymers and include those peptides of...

...from 1 to 100 and preferably from 1 to 10.
It is also known that *LPS* binding monoclonal antibodies (anti-*LPS* antibodies) may be prepared which recognize epitopes in the core region of the *LPS* molecule and are cross-reactive and cross-protective against endotoxicosis caused by Gram-negative bacteria.
These anti-*LPS* antibodies bind to the antigenic determinants of the *LPS* core of different genera of Gram-negative bacteria where the core consists essentially of an oligosaccharide region bound to Lipid A but not involving the specific O-carbohydrate region of *LPS*. Said antibodies:
a) bind to the *LPS* core structure from at least one species of Gram-negative bacteria and from each of the genera of Escherichia, Salmonella and Pseudomonas *LPS*; and
b) are effective in treating clinical manifestations of infection in a mammalian host caused...

...quantity.

SUMMARY OF THE INVENTION
The present invention is based on the combined use of *LPS* binding peptides (specific for the Lipid A region) and *LPS* binding antibodies (specific for the core region), to detoxify *LPS* in vivo and in vitro. The detoxification of *LPS* provides an approach to the prophylaxis and treatment of septic shock as well as the removal of *LPS* from substrates that are prepared for infusion into humans and animals.

Accordingly, it is a...of this invention to provide novel antigenic and non-toxic complexes of Lipid A or *LPS* with a peptide and an *LPS* recognizing antibody with different molecular specificities.

It is also an object of this invention to provide a method of producing novel non-toxic Lipid A or *LPS* antigens.

In particular, it is object of the present invention a pharmaceutical composition which comprises:

(1) an *endotoxin* binding peptide which is a monomer, linear polymer, cyclic monomer or cyclic polymer of formula...

...of 2; and

(2) an antibody specifically binding to the antigenic determinants present in the *endotoxin* core of different genera of Gram-negative bacteria, where the core consists essentially of Lipid...

...and of the immediately adjacent core oligosaccharide covalently bound to the Lipid A of the *endotoxin*, wherein said antibody:

(a) binds to the *endotoxin* core from at least one species of Gram-negative bacteria from each kind of *endotoxin* oligosaccharide core region produced by Escherichia, Salmonella, Pseudomonas, Klebsiella and Neisseria.

It is also an...

...and/or treatment of endotoxicosis and septic shock.

Physiological conditions other than septic shock where *LPS* is the principal cause may be treated by the combined use of peptides and anti-*LPS* antibodies of this invention. These conditions include, but are not limited to, bacterial meningitis, viral...

...will become apparent on reading the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The *LPS* binding and neutralizing peptides are described in U.S, Patent Nos. 5,358,933 and...

...PCT/Publication No. WO95/03327, the contents of which are incorporated into this description.

Known *LPS* binding monoclonal antibodies may be produced using procedures described by Kohler and Milstein, Nature, 256:495, 1975. Certain *LPS* binding antibodies are described in U.S. Patent Nos. 5,057,598 and 5,179...

...incorporated into this description by reference.

As the binding specificity characteristics are different for anti-*LPS* peptides and anti-*LPS* antibodies, the two molecular entities can be administered at the same time in admixture or...

...treatment of endotoxicosis and septic shock in humans or mammals in general. The dose of *SAEP* in humans can vary from 0.1 (mu)g to 2 mg/kg of body...

...intravenously in a single or multiple dose on a daily basis. The dose of anti-*LPS* antibodies may vary depending on the severity of the patient's condition. The antibodies can...

...mu)g and 15 mg/kg of body weight. For in vitro use to detoxify *LPS*, a dose may be used which is similar to the aforestated dose for in vivo...

...the host. Those skilled in pharmacology may ascertain the proper dose using standard procedures.

The *SAEPs* and the anti-*LPS* antibodies may be administered intravenously or parenterally using well known pharmaceutical carriers or inert diluents...

...enzymes of the alimentary tract. Water or isotonic saline solution are preferred diluents for an *SAEP* or antibody concentration of between 0.01 and 1 mg/ml.

The invention also includes the detoxifying use of the *SAEP* and anti-*LPS* antibody in systems containing *LPS* dispersed in a fluid.

This procedure may be used to detoxify pharmaceuticals such as vaccines ...

...the two molecules as additives for fluids which can develop bacterial microbic growth with consequent *LPS* production. In this respect, the presence of the non-toxic compositions of the invention will detoxify any *LPS* which is produced following bacterial contamination.

CLAIMS 1. A pharmaceutical composition which comprises:

(1) an *endotoxin* binding peptide which is a monomer, linear polymer, cyclic monomer or cyclic polymer of formula...

...of 2; and

(2) an antibody specifically binding to the antigenic determinants present in the *endotoxin* core of different genera of Gram-negative bacteria, where the core consists essentially of Lipid...

...and of the immediately adjacent core oligosaccharide covalently bound to the Lipid A of the *endotoxin*, wherein said antibody:

(a) binds to the *endotoxin* core from at least one species of Gram-negative bacteria from each kind of *endotoxin* oligosaccharide core region produced by Escherichia, Salmonella, Pseudomonas, Klebsiella and Neisseria.

2. A pharmaceutical composition...

...n ranges of from 1 to 10.

3. A pharmaceutical composition which comprises:

(1) an *endotoxin* binding peptide which is a monomer, linear polymer, cyclic monomer or cyclic polymer of formula...

...of 2; and

(2) an antibody specifically binding to the antigenic determinants present in the *endotoxin* core of different genera of Gram-negative bacteria, where the core consists essentially of Lipid...

...and of the immediately adjacent core oligosaccharide covalently bound to the Lipid A of the *endotoxin*, wherein said antibody:

(a) binds to the *endotoxin* core from at least one species of Gram-negative bacteria from each kind of *endotoxin* oligosaccharide core region produced by Escherichia, Salmonella, Pseudomonas, Klebsiella and Neisseria,

for use as a medicament.

4. A pharmaceutical composition which comprises:

(1) an *endotoxin* binding peptide which is a monomer, linear polymer, cyclic monomer or cyclic polymer of formula...

...of 2; and

(2) an antibody specifically binding to the antigenic determinants present in the *endotoxin* core of different genera of Gram-negative bacteria, where the core consists essentially of Lipid...

...and of the immediately adjacent core oligosaccharide covalently bound to the Lipid A of the *endotoxin*, wherein said antibody:

(a) binds to the *endotoxin* core from at least one species of Gram-negative bacteria from each kind of *endotoxin* oligosaccharide core region produced by Escherichia, Salmonella, Pseudomonas, Klebsiella and Neisseria,

for use in the...

2/3,KWIC/5 (Item 1 from file: 388)
DIALOG(R)File 388:PEDS: Defense Program Summaries

00004873

Defense Research Sciences

Binder: PROGRAM ELEMENT DESCRIPTIVE SUMMARY - FY1997
Service: DEFENSE AGENCIES
Pub. Date: July 23,1996
Source: Forecast International/DMS
Language: ENGLISH
Word Count: 14883
Pgm.Element: 0601102A

Country: UNITED STATES
Industry: AEROSPACE AND DEFENSE
Binder Code: PEDS1997

...for mustard and sarin and evaluate biosurfactant/nutrient addition treatments for remediation of APG and *SAEP* soils.

- 16 -Revised economic assumptions not available for

-Identify means to produce subunit (pilus, capsule or *LPS* conjugate) macromolecules as potential gonorrhea vaccines;

identify monoclonal antibodies against wound infecting bacteria that protect...

...protection in animal models of sepsis with antisera from animals immunized with E. coli J5 *lipopolysaccharide*.

2/3,KWIC/6 (Item 2 from file: 388)
DIALOG(R)File 388:PEDS: Defense Program Summaries

00004161

Defense Research Sciences

Binder: PROGRAM ELEMENT DESCRIPTIVE SUMMARY - FY1996
Service: ARMY
Pub. Date: October 5,1995
Source: Forecast International/DMS
Language: ENGLISH
Word Count: 14706
Pgm.Element: 0601102A

Country: UNITED STATES
Industry: AEROSPACE AND DEFENSE
Binder Code: PEDS1996

...for mustard and sarin and evaluate biosurfactant/nutrient addition treatments for remediation of APG and *SAEP* soils. (998)

FY 1997 Planned Program:
Synthesize cyclic nitramine using enzymatic methods. (1677)
omplete all...resistance genes, identify drug resistance mechanisms. (2071)
Identify means to produce subunit (pilus, capsule or *LPS* conjugate) macromolecules as potential gonorrhea vaccines; identify monoclonal antibodies against wound infecting bacteria that protect...

...FY 1996 Planned Program:
Explore and exploit feasibility to treat septic shock with antibodies to *lipopolysaccharide*. (517)
Identify feasible formulations for local hemostatic agents; fibrin glues, hematinics, chitin products, etc. (1493...
?ds
Set Items Description
S1 15 (SAEP? AND (LPS OR ENDOTOXIN? OR LIPOPOLYSACCHARIDE?))
S2 6 REMOVE DUPLICATES S1 (unique items)